IMPLICATIONS OF SYMBIOTIC NITROGEN FIXATION BY DESERT PLANTS

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ABSTRACT.— There is little information available regarding symbiotic nitrogen fixation by desert plants, particularly with respect to the group of nodulated non-legumes. Early investigations of nodules on non-legumes were contemporary with early studies of the Leguminoseae; however, comparatively little is known even now regarding the extent to which non-legumes fix nitrogen.

A review of literature regarding changes in concepts of nitrogen fixation by legumes and nodulated non-legumes is presented.

Evidence from recent studies on nodulated non-legumes, including those in desert areas, indicates some 31 species of 21 genera in 12 new or previously unrecognized plant families show nodulation with implications of nitrogen fixation.

Some biological aspects of symbiotic nitrogen fixation are discussed, and reports in the literature of nitrogen fixation in natural plant populations are reviewed.

Nitrogen fixation reactions in the desert environment are considered in terms of the areas of investigation needed to help define (1) the magnitude of nitrogen fixation, (2) the extent of nodulation in non-legume plants, and (3) factors affecting this nodulation in the desert ecosystem.

The ability to understand any phenomenon is often, as Goethe once wrote, "we see only what we know." This applies very well to what is known about symbiotic nitrogen fixation by desert plants, particularly for that group of plants referred to as nodulated non-legumes.

Early invesigations of root nodules on non-leguminous Angiospermae were contemporary with studies on the Leguminosae. In fact, studies on both groups of plants were often conducted by the same investigators (Allen and Allen 1965). A general lack of interest or familiarity with the non-legumes, in contrast to the interest in legumes of agricultural and economic importance, has been largely responsible for the delay in studying nodulated non-legumes. Other factors contributing to this delay are: (1) difficulty in isolating the causative endophyte, (2) difficulty encountered in bringing about reinfection, (3) organisms or endophytes responsible for infection not fitting into any one specific group of organisms as in the case of the legumes, and (4) lack of understanding about environmental and other conditions responsible for the induction of infection.

Until recently little had been accomplished in discovering new plant families and species or determining their role in symbiotic nitrogen fixation. Interest in the desert plants was stimulated by the discovery of nodulelike structures on the roots of an herbaceous western sage, *Artemisia ludoviciana* of the Compositae (Farnsworth and Hammond 1968). Nodules were also observed on the roots of the desert prickly pear, *Opuntia fragilis* of the Cactaceae. These observations encouraged further study of nitrogen fixation by desert vegetation. Apparent root-nodule symbiosis in two species of desert plants was found by Wallace and Romney (1972) and acetylene reduction, suggesting nitrogen fixation, in root systems of several other species. The enzymatic activity of root nodules of two species of the Compositae, *Artemisia ludoviciana* and *A. michauxiana*, was found to reduce acetylene (Farnsworth and Clawson 1972; Clawson 1973). The above findings suggest that symbiotic nitrogen fixation may be an important process in the nitrogen cycle of desert ecosystems.

HISTORICAL REVIEW

Nodulation of Legumes

Although the importance of legumes was recognized in ancient agriculture (Waksman 1927; Fred et al. 1932; Nutman 1965), their significance in the role of symbiotic nitrogen fixation was not discovered until more than two thousand years later. The understanding of the phenomenon of symbiotic nitrogen fixation has evolved slowly, but in specific steps, during the past nearly 400 years

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since nodules were first reported to have been observed on certain species of the legumes.

Waksman (1927) cites Malpighi as the first to refer to nodules on legumes:

> The presence of nodules on the roots of leguminous plants was recorded by Malpighi as early as 1687, but he as well as others, considered them as root galls.

Burrill and Hansen (1917) cite Malpighi's paper but do not report any of his findings. Neither Waksman nor Burrill and Hansen refer to an earlier paper of Malpighi in 1679 (Fred et al. 1932), in which he referred to the "swellings on the roots of leguminous plants as galls caused by insect larvae."

Fred et al. (1932) recognize Fuchs as the first to observe nodules on the roots of leguminous plants. In 1542 he pictured nodules on the roots of four legumes. They also make two rather significant statements:

> (1) The question of who first observed nodules on the roots of leguminous plants provokes more curiosity than can be easily satisfied. If we are to judge from the first published pictures of nodules which we have been able to find, we must give the credit to Fuchs, and (2) Nearly a hundred years later, 1679 . . . Malpighi referred to the swellings on the roots of leguminous plants as galls caused by insect larvae.

His bibliography lists 17 references to legume nodulation from 1687 to 1860, and 389 references from 1860 to 1916. Also listed are 63 references to nodulated non-legumes between 1860 to 1915. Waksman also pointed out that in 1858 Lachmann observed that motile bacteria cause the formation of nodules on legumes and suggested that this might be effective in nitrogen fixation. He also made reference that Woronin in 1866 found bacteria in the nodules but considered them to be pathological outgrowths.

Fred, Baldwin, and McCoy (1932) presented a comprehensive bibliographical study on nodulation, including many early references not mentioned by Burrill and Hansen or Waksman. In the preface they refer to "three major attempts to review the more important papers on root nodule bacteria" as (1) 1889-1890 by Frank, (2) 1894-1903 by McDougal and Schneider, and (3) 1911 by Smith. They also state:

> Many scientific papers have appeared since then treating in detail various phases of the root nodule problem; yet not one of them fully covers the subject.

A major breakthrough in symbiotic nitrogen fixation came in 1884-1886 when Hellriegel, Wilfarth, and Atwater (Waksman 1927; Burrill and Hansen 1932) demonstrated that nodules on legumes were caused by a bacterial organism and that it was within these nodules that the bacteria fix atmospheric nitrogen. The causative organism was isolated in pure culture by Beijerinck in 1888 and named by him as *Bacillus radicicola* but later changed to *Rhizobium leguminosarum* (Waksman 1927).

Table 1 is a summary of the main periods of investigation and concepts of nodulation of legumes.

By 1890 the significance of nitrogen fixation in leguminous plants had been firmly established. The application of this phenomenon to agriculture proved to be of such economic importance that interest in this area far overshadowed the study of nodulated non-legumes. Investigation of the phenomenon in non-legumes was virtually discontinued for approximately 60 years.

Nodulated Non-legumes

The unusual nodules on the roots of the alder, *Alnus glutinosa*, Betulaceae, attracted the attention of Meyen as early as 1829 and of Woronin in 1866 (Fred et al. 1932). Nodulation of alder was also reported by Von Jager in 1860 (Fred et al. 1932) but he, as did many early workers on nodulated legumes, suggested that they were insect galls.

By early 1897 nodulation had been reported on many species of four additional plant families, including the Elaeagnaceae in 1876 by Warming, Myricaceae in 1886 by Brunchorst, Rhamnaceae in 1890 by Beal, and the Casuarinaceae in 1897 by Janse (Fred et al. 1932). This brought to five the number of plant families of nodulated non-legumes recognized prior to 1900.

These non-leguminous plants were predominantly forest trees and other woody plants of much less economic importance than legumes. The fact that nodulation was reported in only one new plant family, Coriariaceae, during the next 50

TABLE 1. Major periods of nodule investigation and concepts regarding formation and function.

	Period I	Reference
Ancient agriculture		
B.C. to scientific agriculture		Waksman 1927
Legumes "invigorate" and "manure"		Fred et al. 1932
Soil		Nutman 1965
	Period II	
Nodules reported on legumes		
1542	Fuchs pictured nodules on four species of legumes	Fred et al. 1932
1556	Bock pictured nodulation	Fred et al. 1932
1586	Dalechampes reported outgrowths as "tubercles" but normal outgrowths	Fred et al. 1932
1679	Malpighi—galls caused by insect larvae	Fred et al. 1932
1687	Malpighi—root galls	Waksman 1927
	Period III	
Concept of nitrogen fixation		
1858	Lachman observed motile bacteria; normal outgrowths for storage suggested nitrogen fixation	Waksman 1927
1866	Woronin observed bacteria but considered nodules pathological	Waksman 1927
1879	Frank demonstrated nodules caused by outside infection of bacteria	Waksman 1927
1886	Hellriegel-Wilfarth-Atwater demonstrated nitrogen fixation	Waksman 1927 Fred et al. 1932
1888	Beijerinck isolated causative organism in pure culture	Waksman 1927 Fred et al. 1932
	Period IV	
Twentieth century research		

years is evidence that interest in this group of plants had greatly diminished. In 1930 Katoaka reported nodules on the roots of *Coriaria japonica* (Fred et al. 1932). Evidence of the occurrence of nodules on many different species of the six known families was reported during this period.

The literature contained conflicting reports relative to the endophyte or causative agent of the nodulation of non-leguminous plants. In an attempt to resolve some of these conflicts, the work of Hawker and Fraymouth (1951) was undertaken. Their work stimulated a renewed interest, almost worldwide, in the nodulated non-legumes as important contributors of nitrogen to different ecosystems. Some extent of this interest is shown in reviews by Allen and Allen (1958, 1965), Becking (1970), Bond (1958, 1963), Silver (1971), and Stewart (1966, 1967). Bond and co-workers in Glasgow are to be complimented for their lead in physiological studies of nodulated non-legume plants and their present involvement in a compilation of current information worldwide on this group of plants.

Between 1951 and 1965 two new plant families were added to the list. The first was Rosaceae, after the observation of nodules on the roots of *Dryas drummondii* by Lawrence in 1953 (Bond 1963) and by Crocker and Major in 1955 (Allen and Allen 1965). In 1964 Allen and Allen found nodules on *Arctostaphylos uva-ursi* of the Ericaceae (Allen and Allen 1965).

By 1971 more than 200 species in 13 genera of eight non-leguminous plant families were recognized to nodulate (Table 2). To this list Farnsworth and Hammond (1968) added Compositae and Cactaceae (Youngberg and Wollum II 1970).

We also note that Beijerinck in his work in 1888 reported nodules on the roots of two species of Scrophulariaceae,

TABLE 2. Families and genera (with some species[1]) of Angiospermae recognized as nodulated nonlegumes as combined from several reviews and references.

Family/Species[1]	Incidence[2]	Early investigators	References[3]
Betulaceae			
Alnus glutinosa	25/35	1829 Meyen	Fred et al. (1932)
glutinosa	(25)	1866 Woronin	Burrill & Hansen (1917); Fred et al. (1932); Allen & Allen (1965)
glutinosa		1896 Hiltner	Fred et al. (1932); Burrill & Hansen (1917); Bond (1963)
glutinosa		1898 Hiltner	Fred et al. (1932); Bond (1963); Allen & Allen (1965)
Casuarinaceae[4]			
Casuarina muricata	14/45 (35)	1897 Janse	Fred et al. (1932); Allen & Allen (1965)
		1915 Kamerling	Fred et al. (1932)
equisetifolia		1918 Miche	Fred et al. (1932); Bond (1963)
triangularis		1933 Mowry	Allen & Allen (1965); Bond (1963); Becking (1970)
Coriariaceae[4]			
Coriaria japonica	12/15 (10)	1930 Katoaka	Fred et al. (1932); Allen & Allen (1965); Bond (1963); Becking (1970)
arborea		1958 Harrison & Morrison	Fred et al. (1932); Bond (1963); Allen & Allen (1965)
myrtifolia		1958 Bond	Bond (1958); Bond (1963); Allen & Allen (1965); Becking (1970)
Elaeagnaceae			
Elaeagnus	9/45 (30)	1876 Warming	Burrill & Hansen (1917); Fred et al. (1932)
Hippophae		1876 Warming	Burrill & Hansen (1917); Fred et al. (1932)
Shepherdia		1876 Warming	Burrill & Hansen (1917); Fred et al. (1932)
Elaeagnus pungens		1886 Brunchorst	Burrill & Hansen (1917); Fred et al. (1932); Allen & Allen (1965)
angustifolia		1892 Nobbe, Hiltner et al.	Burrill & Hansen (1917)
angustifolia		1898 Hiltner	Burrill & Hansen (1917); Fred et al. (1932); Bond (1963)
argentia		1910 Arzberger	Fred et al. (1932)
Hippophae rhamnoides	1/1 (2)	1934 Roberg	Bond (1963); Allen & Allen (1965); Becking (1970)
Shepherdia argentia	2/3	1910 Warren	Allen & Allen (1965)
canadensis	(3)	1957 Gardner & Bond	Bond (1958); Allen & Allen (1965); Becking (1970)
Ericaceae			
Arctostaphylos uva-ursi	1/40 (0)	1964 Allen et al.	Allen & Allen (1965); Becking (1970)
Myricaceae			
Myrica (Comptonia)	12/35 (45)	1886 Brunchorst	Burrill & Hansen (1917); Fred et al. (1932)
		1890 Moeller	Burrill & Hansen (1917); Fred et al. (1932)
sepida		1902 Chevalier	Allen & Allen (1965)
rubra		1902 Shibata	Burrill & Hansen (1917); Bond (1963); Allen & Allen (1965)
asplenfolia et al.		1910 Arzberger	Burrill & Hansen (1917); Allen & Allen (1965)
gale		1911 Bottomley	Burrill & Hansen (1917); Fred et al. (1932)
gale, *cerifera* et al.		1919 Youngen	Bond (1963); Allen & Allen (1965)
Rhamnaceae			
Ceanothus americanus	30/55 (40)	1890 Beal	Burrill & Hansen (1917); Fred et al. (1932); Becking (1970)
		1910 Arzberger	Burrill & Hansen (1917); Fred et al. (1932); Allen & Allen (1965)
Discaria toumatou	1/10	1958 Morrison & Harris	Bond (1963); Becking (1970)
		1961 Morrison	Allen & Allen (1965); Becking (1970)

(Table 2 continued)

Rosaceae				
Dryas drummondii	3/4 (0)	1953	Lawrence	Bond (1963) p. 72; Becking (1970)
drummondii		1955	Crocker & Major	Allen & Allen (1965); Becking (1970)
Purshia tridentata	2/2 (0)	1961	Wagle & Vlamis	Allen & Allen (1965); Becking (1970)
Cerocarpus betuloides	1/20 (0)	1964	Vlamis et al.	Allen & Allen (1965); Becking (1970)

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[1] Only principal species upon which the very early investigations were made of each genus are listed here.

[2] *Incidence* refers to the ratio of species upon which nodules have been found to the number of known species as reported by Silver (1971). Numbers in parenthesis are numbers of nodulated species as indicated by Bond (1963).

[3] Reference here is made only to those early investigators of specific species in each genus. This is in order to avoid duplication of the same reference by many authors, i.e. Allen, Becking, Bond, Nutman, Silver, Stewart, and others. Less than 20 percent of the nodulated species are therefore referenced.

[4] Families not indigenous to North America.

Melampyrum pratense and *Rhinanthus major* (Mishustin and Shil'nikova 1968; English ed. 1971).

A difference of opinion appears to exist with regard to nodulation in the Zygophyllaceae. Nodules were first observed on the roots of *Tribulus terrestris* by Isachenko in 1913 on plants growing in the sands of Southern Bug, in south Russia (Mishustin and Shil'nikova 1968). Nodules were later reported by Sabet (1946) on the roots of another species of *Tribulus*, as well as other Zygophyllaceae, including *Zygophyllum album*, *Z. decumbens*, *Z. simplex*, *Fagonia arabica*, and *Tribulus alatus*. This nodulation occurred on plants growing on poor sandy soils of the Egyptian deserts. Sabet also reported finding a bacterial endophyte very similar to that of legumes in nodules of *Z. coccineum*. Shemakhanova and Mishustin (1966) reported nodulation of several species in the Zypophyllaceae as well as nodulation on the roots and leaves of several other plant families.

Allen and Allen (1950) observed swellings or nodular growths on the roots of *Tribulus cistoides* but found no evidence of bacterial infection. They concluded that this was probably "false nodulation," or "merely accessory starch storage areas," and "accordingly, this species does not warrant inclusion in the list of those bearing nodules." The plants studied had been grown in a greenhouse in Wisconsin in unsterilized soil from seeds imported from Haiti. They do not report having inoculated this soil with soil from Haiti or Egypt, nor do they report having attempted to duplicate the environmental conditions of these areas. Montasir and Sidrak in 1952 (Mishustin and Shil'nikova 1968) report that nodules form on the roots of *Z. coccineum* only under conditions of high temperature and low moisture. High levels of soil moisture impede the formation of nodules.

Mostafa and Mahmoud (1951) reported isolating bacterial organisms from the root nodules of *Z. coccineum*, *T. alatus*, and *F. arabica*. They concluded that the endophyte was a *Rhizobium* sp. since the organisms from *Tribulus alatus* crossed effectively with *Trifolium alexandrinum* (Egyptian clover) and *Arachis hypogoea* (peanut). The organisms isolated from *Z. coccineum* also produced nodules on the roots of *Arachis hypogoea*.

Athar and Mahmoud (1972) also reported finding nodules on the roots of three species of Zygophyllaceae in West Pakistan: *Tribulus terrestris*, *Fagonia cretica*, and *Zygophyllum simplex*. They concluded that the endophyte isolated from the nodules of *T. terrestris* and *Z. simplix* "belongs to the genus *Rhizobium*."

Farnsworth and Hammond (1968) observed nodules on the roots of a species of western sage, *Artemisia ludoviciana*, sometimes referred to as gray sagewort, herbaceous sagebrush, Louisiana sagebrush, western mugwort, or wormwood, of the Compositae, and on *Opuntia fragilis* prickly pear) of the Cactaceae. Further investigations by Farnsworth and associates, at the Snowberry Exclosure on the Manti-La Sal Forest in Utah (Clawson et al. 1971; Clawson 1973), disclosed nodules also on the roots of *Artemisia michauxiana* and *Chrysothamnus viscidiflorus* of the Compositae. Species of four additional plant families were also found to have nodulated roots: *Mertensia brevistyla* (Boraginaceae); *Castilleja chromosa*

(Scrophulariaceae); *Lomatium triternatum* (Umbelliferae); and *Viola praemorsa* (Violaceae).

Trinick (1973) found nodules on the roots of *Trema aspera* of the family Ulmaceae, and he successfully isolated a bacterial organism which effectively cross-inoculated 4 of 19 species of legumes investigated. This particular endophyte is reported to satisfy the criteria necessary to classify it as a *Rhizobium* sp. This work by Trinick marks the second time an endophytic organism has been demonstrated to cross-inoculate between legumes and a non-leguminous plant, the first being reported by Mostafa and Mahmoud (1951).

These findings help answer the question raised by Burrill and Hansen (1917): Is symbiosis possible between legume bacteria and non-legume plants? Their work, though unsuccessful, was a continuation of Schneider's work in 1893, which attempted to develop a symbiosis between legume bacteria and non-leguminous plants. The great hope was to inoculate cereals and grasses (Gramineae). The following quotes were made by Burrill when introducing the work of Schneider in 1893 (Burrill and Hansen 1917):

> Can the organisms be made to grow upon these roots (grasses or cereals) by artificial means?
>
> It must be confessed that it would have been exceedingly hazardous for anyone to have expressed an affirmative opinion upon this question; but the vast importance of the matter made it desirable to try anything which gave the least promise of success . . . while little direct evidence has been gained in favor of ultimate success, it is desirable to publish an account of the work so far done, with the hope of being able at some future time to add greatly to the information now obtained.

The translation into English of the work of Mishustin and Shil'nikova (1968) has brought to our attention the fact that several plant species among the Gramineae show nodulation. Included are the meadow foxtail, *Alopecurus pratensis*, Kentucky bluegrass, *Poa pratensis*, and siberian lymegrass, *Clinelymus sibiricus* and *C. ventricosus*.

Table 3 is a list of the families and genera of nodulated non-legumes reported in the literature since 1965 or previously reported but not generally recognized.

BIOLOGICAL ASPECTS OF SYMBIOTIC NITROGEN FIXATION

Since the world population explosion has increased the demand for high protein food, mankind must increase biological nitrogen fixation by both legumes and non-legume nitrogen-fixing plants in order to produce the needed quantity and quality of food. There is an immediate need to induce agriculturally important legumes to fix nitrogen more efficiently. Another need is to find other nitrogen-fixing plants, which can be developed as cultivars, and learn how they fix atmospheric nitrogen and contribute to soil fertility.

An extensive literature has developed on the nitrogen-fixing legumes, centered around about 200 species commonly used in agriculture, which contribute from 100 to 200 kilograms of nitrogen per hectare per year (Allen and Allen 1958). Work also has been reported on some non-leguminous plants which have the capacity to fix significant amounts of nitrogen. Significant findings in this area have been reviewed by: Fred et al. 1932; Wilson 1940; White et al. 1953; Allen and Allen 1958; Nutman and Mosse 1963; Bartholomew and Clark 1965; Stewart 1966; Bond 1963, 1967; Mishustin and Shil'nikova 1968; Becking 1970; Lie and Mulder 1971; Postgate 1971.

Legume Symbiosis

More than 12,000 species of legumes are distributed throughout nonagricultural soils. Many are shrubs and trees not yet examined for root nodules or the capacity to fix atmospheric nitrogen. Inasmuch as nearly 90 percent of the legumes so far examined are nodule-bearing, there is reason to suspect that many more species also fix nitrogen and contribute to soil fertility (Allen and Allen 1958).

Donald (1960) has estimated that some 10^8 tons of atmospheric nitrogen are fixed worldwide annually and that most of it comes from symbiotic fixation by legumes growing in natural and cultivated associations. Nutman (1965) listed the following estimates of nitrogen fixed in kg/ha per crop (compiled from data of Spector and Seeger): alfalfa, 50-350; clovers, 50-200; peas, 30-140; peanuts, 88; pastures with legumes, 10-550. The amounts fixed by individual plants are

TABLE 3. Families and genera of nodulated non-leguminous Angiospermae reported since 1965 as being nodulated, or previously reported[1] but not generally recognized.[2]

Family/Species	Early investigators	References
Scrophulariacea		
Melampyrum pratense	1888 Beijerinck	Mishustin & Shil'nikova (1968)
Rhinanthus major	1888 Beijerinck	Mishustin & Shil'nikova (1968)
Castilleja chromosa	1972 Farnsworth & Clawson	Clawson (1972; 1973)
Zygophyllaceae		
Tribulus terrestris	1913 Isachenko	Lange (1966); Mishustin & Shil'nikova (1968)
	1952 Montasir & Sidrack	Lange (1966); Mishustin & Shil'nikova (1968)
	1972 Athar and Mahmoud	Athar & Mahmoud (1972)
Zygophyllum album	1946 Sabet	Sabet (1946)
Zygophyllum coccineum	1946 Sabet	Sabet (1946); Mostafa & Mahmoud (1951)
	1952 Montasir & Sidrack	Lange (1966); Mishustin & Shil'nikova (1968)
Zygophyllum decumbens	1946 Sabet	Sabet (1946)
Zygophyllum simplex	1946 Sabet	Sabet (1946); Athar & Mahmoud (1972)
	1972 Athar & Mahmoud	
Fagonia arabica	1946 Sabet	Sabet (1946); Lange (1966); Mostafa & Mahmoud (1951)
Tribulus alatus	1946 Sabet	Sabet (1946); Lange (1966); Mostafa & Mahmoud (1951)
Tribulus cistoides	1949 Allen & Allen	Allen & Allen (1950); Mishustin & Shil'nikova (1968)
Fagonia cretica	1972 Athar & Mahmoud	Athar & Mahmoud (1972)
Rubiaceae		
Coffea rubusta	1932 Steyaert	Lange (1966); Mishustin & Shil'nikova (1968)
Coffea klainii	1932 Steyaert	Lange (1966); Mishustin & Shil'nikova (1968)
Gramineae		
Alopecurus pratensis	1938 Nogtev	Mishustin & Shil'nikova (1968)
	1939 Mudrova	Mishustin & Shil'nikova (1968)
Poa pratensis	1958 Savel'ev et al.	Mishustin & Shil'nikova (1968)
Clinelymus sibiricus	1958 Savel'ev et al.	Mishustin & Shil'nikova (1968)
Clinelymus ventricosus	1958 Savel'ev et al.	Mishustin & Shil'nikova (1968)
Cruciferae		
Brassica	1959 Schwartz	Mishustin & Shil'nikova (1968)
Raphanus	1959 Schwartz	Mishustin & Shil'nikova (1968)
Compositae		
Artemisia ludoviciana	1967 Farnsworth	Farnsworth & Hammond (1968)
Artemisia michauxiana	1972 Farnsworth & Clawson	Clawson & Farnsworth (1972); Clawson (1973)
Chrysothamnus viscidiflorus	1972 Farnsworth & Clawson	Clawson & Farnsworth (1972); Clawson (1973)
Artemisia tridentata	1972 Wallace & Romney	Wallace & Romney (1972)
Cactaceae		
Opuntia fragilis	1968 Farnsworth & Hammond	Farnsworth & Hammond (1968)
Opuntia polyacantha	1969 Stutz	Stutz (1969)
Boriaginaceae		
Mertensia brevistyla	1972 Farnsworth & Clawson	Clawson & Farnsworth (1972); Clawson (1973)
Krameriaceae		
Krameria parvifolia	1972 Wallace & Romney	Wallace & Romney (1972)
Umbelliferae		
Lomatium triternatum	1972 Farnworth & Clawson	Clawson & Farnsworth (1972); Clawson (1973)
Violiaceae		
Violia praemorsa	1972 Farnsworth & Clawson	Clawson & Farnsworth (1972); Clawson (1973)
Ulmaceae		
Trema aspera	1973 Trinick	Trinick (1973)

[1] Listed in chronological order in relation to when a species of each family and genus was first reported.

[2] There are still differences of opinion as to whether or not the swellings (nodular growths) on the roots of some of these plants are nodules in the true sense of structure and effectiveness in symbiotic nitrogen fixation. Nodulation has been observed, but the role in specific symbiotic nitrogen fixation of several of these has not yet been shown.

affected by several factors, including the environment, but an effectively nodulated legume can provide itself with all the nitrogen it needs, even when soil nitrogen is deficient. Such also may be the case for nodulated non-legumes in view of findings of MacConnell and Bond (1957) that maximum rates of fixation per unit volume of nodule tissue are similar to those of legumes.

After the legume host dies, the fixed nitrogen becomes available to other plants through decomposition and mineralization processes. Thus, crops in rotation benefit markedly from root nodule symbiosis in agricultural practice. In natural environments non-legumes may receive immediate benefit from legumes growing in close association with them. Even under harsh desert conditions, some species of legumes commonly grow as understory plants, providing nitrogen to non-leguminous trees and shrubs. Wilson (1940) showed evidence that roots of living legumes may excrete appreciable amounts of nitrogen under certain conditions.

The bacteria of all legume nodules are aerobic, gram-negative, non-spore forming rods of the genus *Rhizobium*, which usually have simple nutritional requirements. These nodule bacteria can live freely in soil in the absence of a host plant but, as typical rhizosphere organisms, they are markedly stimulated by plant roots, particularly legumes (Nutman 1965). Bacterial partners of the genus *Rhizobium* were first classified by Fred, Baldwin, and McCoy (1932) into convenient cross-inoculation groups on the basis that members of each group induce nodulation only on certain genera of legumes, and none other. The bacteria appear to show no absolute correlation between nodulation specificity or cultural features; therefore, the only way to determine the species of an isolate is to inoculate a range of leguminous plants.

Nutman (1965) pointed out that the strains of bacteria involved in symbiosis can be distinguished by the number of species of plants which each can infect. The six main cross-inoculation groups are *Rhizobium trifolii*, *R. meliloti*, *R. leguminosarum*, *R. phaseoli*, *R. lupini*, and *R. japonicum*. The clover group nodulated by *R. trifolii* consists only of species of the genus *Trifolium*; the medic (*R. meliloti*) of *Medicago*, *Melilotus* and *Trigonella*; the pea group of *Pisum*, *Vicia*, *Lathyrus* and *Lens*; the bean group of some *Phaseolus* species; the lupin group of *Lupinus* and *Ornithopus*. By contrast the cowpea group contains hundreds of host genera from the *Phaseoleae* and *Genisteae*. Some plant tribes have species in more than one cross-inoculation group, and the genus *Phaseolus* has species in both the bean and cowpea groups. The plant species in each group can be further classified into subgroups on the basis of their nodulation with individual collections of bacterial strains, but these conditions are not within the scope of this discussion.

Inasmuch as microorganisms living in association with plants can fix atmospheric nitrogen, it has long been assumed that the bacteroids in the nodule fix the nitrogen, which then passes on to the host plant, and that the special conditions in the nodule stimulate the bacteroids to perform reactions which they are incapable of doing alone. Symbiotic nitrogen fixation therefore requires certain structures or functions, characteristic of the intact nodule attached to the whole plant, and for which no substitute has yet been found (Nutman 1965). Fixation by excised nodules, or whole excised roots bearing nodules, stops within a few hours after excision (Aprison and Burris 1952; Magee and Burris 1954).

Legumes growing in association with other plant species in the desert environment have a beneficial function in supplying soil nitrogen. Many are annual species which probably are somewhat limited in performing this function due to the shortness of growth season. Inasmuch as the extent of their function in this kind of ecosystem is largely unknown, there is need for additional work to investigate their contribution to maintaining the nitrogen balance in desert soils.

Non-legume Symbiosis

As indicated previously, there has been an increased interest in the symbiotic nitrogen fixation of the non-leguminous plants since 1951. Nodules or nodulelike formations on the roots of non-legumes occur in both angiosperms and gymnosperms. Among the angiosperms, nodules are attached mostly on dicotyledons but they have also been reported on some monocotyledons. In most cases nodules form on the roots but are sometimes found on the leaves of herbaceous plants.

NITROGEN FIXATION IN GYMNOSPERMS.—Root nodules of gymnosperms are found in representatives of the orders Cycadales and Coniferales. The order Cycadales has one family, Cycadaceae, which includes nine genera; root swellings are found on the roots of some 90 species belonging to eight of these genera (Allen and Allen 1965). The Allens report that organisms causing nodules to form in this family are blue-green algae. The nature of the agent and the function of the nodules in the Cycadaceae has not been established; but there is clear evidence of nitrogen fixation (Douin 1954; Bond 1959; Bergersen et al. 1965). The microbial partner occurs in a distinct air space in the nodule cortex of such cycads as *Macrozamia* and *Encephalartos* (Stewart 1967).

Among the 11 genera of the Coniferales known to contain root nodules, the most studied is the genus *Podocarpus*. Nodules are found in 26 species of this genus (Mishustin and Shil'nikova 1968). The agent of nodule formation is unknown but the symbiont occurs intracellularly in the cortex. Most investigators have observed fungal hyphae in the nodules of *Podocarpus* and Becking (1965) identified them as Phycomycetes. Although it appears that the *Podocarpus* symbiont is a fungus, Silver (1971) advanced the supposition of more than one type of symbiotic association. *Casuarina* has been reported to contain mycorrhiza as well as the more familiar actinomycetous root nodules (Silver 1971), and Morrison and English (1967) have described two microsymbionts in *Agathis australis*. So far sufficient work has not been done to show the importance of nodules to the podocarps. The nodules have been reported to fix atmospheric nitrogen; however, there have been reports also of no fixation or of only very moderate fixation (Bond 1959; Bergerson and Costin 1964; and Becking 1965).

NITROGEN FIXATION IN ANGIOSPERMS.—There are 13 genera of eight families of Angiosperms shown and recognized before 1965 to possess root nodules capable of nitrogen fixation (Lange 1966; Bond 1967) (Table 2). All are dicotyledonous woody trees and shrubs. The nodulated genera and the ratio of species bearing nodules to the known species of each genus from the adaption by Silver (1971) are also listed in Table 2. Ecologically these woody perennials are characteristic pioneer plants of areas low in combined soil nitrogen. Nodules occur laterally on the roots as perennial-coralloid masses ranging from a few millimeters to several centimeters in diameter (Stewart 1967). Bond (1963) described long-term experiments comparing the ability to accumulate nitrogen by nodulated and nodule-free plants. In all cases nodulated plants grew well and, in rapidly growing species such as *Alnus glutinosa*, as much as 300 milligrams of nitrogen per plant accumulated in the first season of growth from seed. After exhausting seed nitrogen, the nodule-free plants made essentially no further growth. Bond further pointed out the existence of an irrefutable body of existence supporting claims of nitrogen fixation associated with several species of nodulated non-legumes.

The nature of the endophyte involved can be inferred from indirect evidence in spite of the failure of attempts to isolate pure cultures. Reports of early investigators indicated the probable involvement of either fungi or bacteria, or both, but this generally has not been supported by more recent studies. Silver (1971) pointed out that both light microscopy and electron micrographs of infected roots of *Myrica gale* (Fletcher 1955), *Myrica cerifera* (Silver 1964), and *Alnus glutinosa* (Becking et al. 1964) reveal filamentous structures characteristic of actinomycetes. The best evidence that the endophyte is an actinomycete is the morphology work *in situ* of Becking (1970) who noted three morphological forms of the endophyte: 0.5, μm hyphae, larger (3-4, μm) vesicles, and 0.5-1.0 μm bacterialike cells which packed the host cell. Since the hyphae contained mesosomes and lacked a nuclear membrane, the endophyte cannot be fungal. Bond (1963) indicated that the basic physiology of the nodule is similar to that of legumes; however, detached nodules fixed nitrogen considerably longer than did legume nodules, suggesting the possibility that certain nonlegume endophytes might fix nitrogen in the free state. Allen and Allen (1965) concluded that non-legumes have the capacity to contribute as much nitrogen in proportion to soils in which they grow as do legumes.

Several lines of evidence indicate that root nodules on non-legumes are sites of nitrogen fixation: (1) the high nitrogen contents of nodules compared to roots and shoots of host plants, (2) the inability of non-nodulated tissue to fix nitrogen, and (3) the distribution of ^{15}N following exposure of the root system to an ^{15}N-enriched atmosphere (Silver 1971). The work of Bond (1958) showed that, in all species studied, the ^{15}N enrichment was greater in root nodules than in roots or shoots. *Alnus* nodule tissue was more than eight times as enriched as was root tissue. The lack of activity in denodulated plants and the high activity in excised nodules attests to the nodule being the site of symbiotic fixation.

Ecological Implications

Natural Plant Populations That Fix Nitrogen

Crocker and Major (1955) noted the abundance of nitrogen-fixing *Alnus* in recently deglaciated areas in Glacier Bay, Alaska, and described their ecological importance in plant succession and soil formation. One important feature was the acidifying effect of the alder in lowering the surface soil pH from 8.0 to 5.0 within a period of about 35 years. Similar ecological indications led to tests with *Shepherdia*, another nodulated angiosperm of this area which fixes nitrogen. In these associations they estimated a mean annual rate of nitrogen accumulation of about 60 kg per hectare.

Lawrence (1958) and Lawrence et al. (1967) reported the presence of fleshy, coralloid structures similar in appearance to the nodules of *Alnus* species on plants of *Dryas drummondii*, *D. octopetala*, and *D. integrifolia* growing in recently deglaciated areas at Glacier Bay. It was observed that, after many years of growth, these plants produced disc-shaped mats as large as six meters in diameter which appeared as green islands in a yellow matrix of nitrogen deficient, non-nodulated species.

The work in Canada by Daly (1966) indicated that soil nitrogen may be increased at rates in excess of 160 kg per hectare per year under *Alnus rugosa*. Zavitkovski and Newton (1968) conducted studies which led to estimates that nitrogen fixation rates of as much as 100 kg per hectare per year could be expected from alders during optimal conditions in the field. Since the typical location for many *Alnus* species is along the margins of rivers and lakes, Bond (1967) pointed out the possibilities of substantial contributions of fixed nitrogen to the water. Goldman (1961) investigated nitrogen sources for a lake in California fringed on one side by nodulated trees of *Alnus tenuifolia*. Analysis of soil and water samples at various points around the lake shore disclosed that the alders were an important source of nitrogen to the lake ecosystem.

A study by Dommergues (1963) of sand dunes at Cape Verde planted with *Casuarina equisetifolia* showed an accumulation of some 58 kg of nitrogen per hectare annually in planted areas. Stewart and Pearson (1967) studied the growth of *Hippophae rhamnoides* on dunes on the English coast. In addition to ^{15}N tests which showed active symbiosis, soil and plant analysis indicated total nitrogen accretions of up to 180 kg per hectare per year. Ruth Gadfill (1971) recently reported findings indicating potential benefits of *Lupinus arboreus* as a nitrogen source for young *Pinus radiata* trees in coastal sand dune forestry areas in New Zealand.

The findings from Schramm's (1966) studies of revegetation on anthracite waste spoils in Pennsylvania disclosed that *Comptonia peregrina* (*Myrica asplenifolia*) was an excellent colonizer species under conditions where nitrogen deficiency in soil was a deterrent to plant growth. This is attributable to nitrogen fixation from these nodulated plants. *Alnus glutinosa* also grew successfully on these sites. Detached root nodules from the field showed strong evidence of nitrogen fixation in labelled nitrogen tests conducted by Sloger and Silver (1965). Acetylene reduction assays of nodular tissue of a single *Myrica cerifera* plant in the field in Florida soil indicated a minimal fixation of about 3 kg of nitrogen per hectare per year (Silver and Magne 1970).

Ceanothus species were demonstrated by Delwiche, Zinke, and Johnson (1965), through labelled nitrogen studies, to fix nitrogen. The rates obtained ranged from 10 to 212 mmoles per hour per gram fresh weight in 12 different species. Tomato plants grown in soil which had previously supported *Ceanothus leucodermis* were shown by Green to have about twice the nitrogen content of tomatoes grown in control soil (Hellmers and Kelleher 1959). In nitrogen deficient California soils, Vlamis, Schultz, and Biswell (1958) reported that *C. integerrimus* grew just as well as other plants which had been fertilized with supplemental nitrogen.

The reports of nodules on roots of Gramineae plants and subsequent work by Russian investigators is of ecological significance (Mishustin and Shil'nikova 1968). The species reported by them which have been observed to form nodules and give indications of nitrogen fixation are shown in Table 3. In southern Nevada, Wallace and Romney (1972) found an indication of nitrogen fixation by the introduced grass *Bromus rubens* using the acetylene reduction survey method. This annual grass is commonly found as an understory plant of shrub clumps. Dobereiner and associates (1972) reported extensive nitrogenase activity in the rhizosphere of sugarcane and some other tropical grasses but do not report any nodulation of these plants.

In Utah (Farnsworth and Hammond 1968; Clawson and Farnsworth 1972; Farnsworth et al. 1972; Clawson 1973) apparent nodulation has been observed and nitrogenase activity measured, through acetylene reduction, in *Artemisia ludoviciana* and *A. michauxiana*. Apparent nodulation was also reported on one other Compositae, *Chrysothamnus vicidiflorus* (rabbitbrush) and on species of five other plant families, Table 3.

TABLE 4. Production of ethylene acetylene by various root nodule samples.

Sample type	Nanamols ethylene/mg fr wt nodule/day	*Mg fr wt nodule/sample
Alfalfa	229	1364
Red Clover	240	1461
Sweet Clover	247	1354
Artemisia ludoviciana 5/4/72	259	1450
Artemisia ludoviciana 5/11/72	38.8	1375
Artemisia ludoviciana 5/18/72	12.6	1463
Artemisia ludoviciana 5/23/72	.48	1372
Artemisia ludoviciana 5/30/72	.3	1495

*Milligram of fresh weight of nodule

When nodules of *Artemisia ludoviciana* were young and growing in an environment of moist, cool soil, the reduction of acetylene was found to be in the magnitude of that of alfalfa, red clover, and sweet clover. As soil moisture became depleted and nodules became older, acetylene reduction decreased rapidly. These relationships are shown in Table 4.

Nodulation appears to be largely dependent upon soil moisture and soil temperature interrelationships. Soil temperatures at the time of most profuse nodulation on the *Artemisia* sp. were observed to range from 5-10 C. Soil moisture content was greater than one-half of the available range.

In Table 5 data are given of an unpublished study by Farnsworth and associates on the comparative nitrogen content of alfalfa and three desert shrubs.

TABLE 5. Nitrogen content of alfalfa compared with non-leguminous plants.

Plant species	Percent total nitrogen[1]	Precent protein (protein equivalent)
Alfalfa (*Medicago sativa*)	2.4 to 3.0	14.0 to 18.7
Herbaecous sagebrush (*Artemisia ludoviciana*)	3.3 to 3.8	20.6 to 23.7
Rabbitbrush (*Chrysothamnus vicidiflorus*)	4.1 to 4.6	24.6 to 28.7
Greasewood (*Sarcobatus vermiculatus*)	4.9 to 5.4	30.6 to 33.7

[1] Total nitrogen content was determined on 8 to 10 samples of plant material collected in different areas of Utah, Nevada, and Wyoming in early June 1968 and 1969.

Frischknecht (1963) found that forage production of crested wheatgrass when seeded directly in land covered with growth of rabbitbrush (*Chrysothamnus nauseosus*) was almost as high as when the rabbitbrush was removed just shortly before seeding. Production was also much greater than when the crested wheatgrass was seeded on land from which the rabbitbrush had been removed for quite some time. Some of the results of his study are shown in Table 6.

These data suggest that much is to be learned of the desert ecosystem and that further research of possible nitrogen fixation by desert plants is needed.

In a survey of desert plants in southern Nevada, Wallace and Romney (1972) examined some 30 different species for detection of nitrogen fixation by the acetylene reduction method. Several different species gave positive reactions to the acetylene reduction test, during moist spring months, which ceased after the soil was depleted of seasonal moisture with the onset of hot summer weather. Included were: *Artemisia spinescens*, *A. tridentata*, *Hymenoclea salsola* and *Tetradymia canescens* of the Compositae; *Colegyne ramosissima* of the Rosaceae; *Bromus rubens* of the Gramineae; and *Krameria parvifolia* of the Krameriaceae. Three legumes, *Lupinus argenteus*, *Dalea fremontii*, and *Astragalus lentiginosus*, gave positive tests as did lichen samples which were not identified. Although results of this type are qualitative in nature, they serve to indicate associations suspected of some form of symbiosis. Additional studies will be necessary to investigate root nodule potential, causative endophyte involved, and the possibility of symbiotic or freeliving systems involved in the reactions. Certainly under desert conditions the seasonal variation in moisture and temperature plays an important role in the function of this important life-support process.

Table 6. Forage production of crested wheatgrass when grown on land areas occupied with rabbitbrush (*Chrysothamnus nauseosus*).

Seeding sequence for crested wheatgrass	Yield of forage k/ha
1. Rabbitbrush removed several years prior to seeding	1,072
2. Rabbitbrush removed prior to seeding	1,818
3. Rabbitbrush not removed (crested wheatgrass seeded in rabbitbrush)	1,734

Nitrogen in Desert Soils and Areas of Needed Research

The nitrogen status in desert soils is extremely fragile because of its dependence upon widely fluctuating environmental factors. Generally the available forms of soil nitrogen are of relatively low concentration, partly due to the sparse levels of organic matter, which is one of the results of low precipitation. Concentrations of nitrogen usually are higher in the root zone underneath shrubs than in bare soil areas between shrubs (Nishita and Haug 1973; Wallace and Romney 1972; Romney et al. 1973, 1974).

Even though the total amount of soil nitrogen in the desert system is low, due largely to such a sparse vegetal cover, the nitrogen content in the early spring in the new leaves of some desert plants is unusually high (Romney et al. 1973). It would seem that these particular plants may be nitrogen fixers rather than nitrate accumulators. The analysis for total nitrogen in the new leaves might serve as an indicator of plants involved in nitrogen fixation, whether it be nodulation and direct symbiosis, loose-symbiosis, or loose-association with free-living, nitrogen-fixing organisms.

Soil moisture and temperature largely govern the amounts of nitrogen demanded in annual photosynthetic production and also the amounts subsequently recycled through decomposition and mineralization of litter. Although the soil nitrogen contents are low, one seldom observes symptoms of nitrogen deficiency in foliage of desert vegetation. Its symptomatic appearance might be masked by insufficient seasonal moisture to sustain an amount of vegetative growth that could seriously deplete the supplies of available nitrogen. Furthermore, the sparse stands of vegetation typical of most desert areas do not demand the amounts of nitrogen required by agricultural crops. Most of the nitrogen used by vegetation is subsequently returned to desert soils through leaf fall and litter decomposition, unless the vegetation has been heavily grazed. Leaching, volatilization, denitrification and erosion by rainfall probably account for some loss of soil nitrogen, yet that which is lost by run-off at one site may be compensated by run-in from another site.

Leguminous species are common to some large desert areas, such as those inhabited by mesquite shrubs (*Prosopis* sp.), *Astragalus* or *Lupinus* in association with sagebrush and grasses, but there are vast areas which are virtually devoid of leguminous shrubs. Annual legumes are prevalent in many desert areas during years of favorable moisture; however, the extent of nitrogen input during their few weeks of vegetative growth is not known. Perennial herbaceous legumes probably contribute much more, but they are not generally of widespread distribution. Shrub associations in Mojave Desert areas contain low densities of leguminous shrubs (Wallace and Romney 1972; Romney et al. 1973).

Much has been implied in this paper about host plant symbiotic nitrogen fixation as the source of soil nitrogen. Environmental conditions of the desert ecosystem, however, generally disfavor the widespread occurrence of a form of life support which is dependent upon root nodulation, largely because temperatures and soil moisture are suspected of being of major limiting factors. These fluctuate to extremes in the desert system and specific effects have not yet been determined. Also, the extent to which nitrogen stress is effectively responsible for nodulation and symbiosis is not known.

Nitrogen-fixing bacteria also must not be overlooked as being potentially involved in desert soils when conditions are favorable. Knowles (1965) reported fixation rates by free-living bacteria ranging from 0.1 to 73.0 kg of nitrogen per hectare per year under anaerobic conditions, and up to 34.6 kg annually under aerobic conditions in some Quebec soils. The studies of Yoshida and Ancajas (1973) have demonstrated nitrogen-fixing activities under similar conditions in upland and flooded rice fields.

The concepts of loose-symbiosis, or loose-association, a symbiotic relationship between desert plants and soil microorganisms need to be carefully studied. Raju, Evans, and Seidler (1972) have reported an asymbiotic relationship can exist between corn plants and certain microorganisms. Work of Dobereiner (1972a, 1972b) indicates that this type of relationship exists between sugarcane, tropical grasses, and certain soil microorganisms.

The free-living, nitrogen-fixing blue-green algae are more favorably adapted to function alone or in lichen associations. Nitrogen fixation by lichens is of ecological importance because of their widespread occurrence on desert soils. These associations are probably the most representative of the free-living forms. Fuller et al. (1960) identified many genera of algae obtained from desert soil as blue-green algae, many of which are autotrophic with respect to both carbon and nitrogen. Their ability to fix nitrogen resulted in algae and lichen crusts being four to five times as high in available nitrogen as the soil below. Mayland et al. (1966) found that semiarid desert algae crusts were capable under optimal conditions of fixing nitrogen at rates which are estimated to be equivalent to form one-fourth to one-third of the levels reported to be contributed by blue-green algae in rice paddy fields (Stewart 1966). *Nostoc* species, either alone or in lichen symbiosis, are noted for their capacity to fix atmospheric nitrogen under widely varied environmental conditions.

It should also be pointed out that mycorrhizae are found in the roots of numerous desert plants forming a close union between fungal hyphae and tissues of the host plant (Went and Stark 1968). Because of their widespread occurrence in the decomposing litter underneath desert shrubs, either in ectotrophic or endotrophic forms, the mycorrhiza-forming fungi have the potential to contribute significantly to the nitrogen supply of desert soils. The cycle of development of the fungal endophyte and action upon the host plant is somewhat similar to the corresponding process in the nodulated plant species (Meyer 1966; Mishustin and Shil'nikova 1968).

In the numerous areas of needed research on nitrogen fixation by plants, an understanding of genetic and biochemical compatibility between certain organisms and the specific host plants must be developed.

In conclusion, we marvel that such a tremendous amount of work has been done leading to man's understanding of symbiotic nitrogen fixation, and yet, in the magnitude of the desert ecosystem, with its diversity of plants and its seemingly endless activity, man has spent so little time investigating the role of nitrogen fixation by desert vegetation.

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